3364-Pos Board B92
Solvent Dependent Shift of Fluorescence Properties of Fluorescent Proteins
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Fluorescence properties of fluorophores are known to be affected by various environmental factors (e.g. pH, temperature and permittivity of solvent). By utilizing these properties, we think various fluorescent probes been developed and utilized to monitor the intracellular environment of living cells. The properties of fluorescent proteins (FPs) are also affected by the environment but in a more complicated manner due to their unique chromophore structures and the surrounding β-barrel region. In this study, we investigated how the fluorescent properties of FPs are affected by solvents, and explored the possibility of using them as specific probes to characterize the local environment of a target protein. When purified FPs (BFP, ECFP, EGFP, Venus, mCherry and mRFP) were exposed to various alchols (methanol, 1-propanol, 2-propanol, ethanol, 2,2,2-trifluoroethanol (TFE)), fluorescent intensities were significantly affected. The fluorescence intensities of ECFP, EGFP and BFP, but not Venus, mRFP and mCherry, were dramatically decreased in a solution of more than 30% TFE, whereas a lower concentration of alcohol had almost no effect. The circular dichroism (CD) spectrum of ECFP demonstrated that β-sheets are significantly collapsed in 30% TFE. Molecular dynamics (MD) simulations also demonstrated significant changes in the β-barrel structure and in the accessibility of solvents to the chromophore. The fluorescence life-time measurements demonstrated that the life-time of ECFP in 30% TFE was drastically decreased, suggesting that solvent relaxation occurs. These results suggest that hydrophobic solvent such as TFE first attacks the β-barrel structure of FPs, which then allows solvent molecules to access the chromophore. By combining environment-sensitive and -intensive FPs, we successfully developed FP probes to monitor the local environment of target proteins.

3365-Pos Board B93
Influence of Hofmeister Salts on the Structure, Aggregation, and Unfolding of RECA
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RecA is an *Escherichia coli* protein that catalyzes the strand exchange process involved in DNA repair. Previous circular dichroism (CD) studies in our lab have shown that high salt concentrations stabilize RecA in a reverse anionic Hofmeister series. Here we utilize infrared spectroscopy to further investigate how various Hofmeister salts alter RecA structure, aggregation, and solvation. Infrared studies were performed in water and deuterium oxide. Spectroscopic evidence shows that salts alter the water OH stretch and amide I and amide II vibrations arising from the protein backbone. Our data suggests salt specific influences on RecA aggregation, secondary structure, and unfolding. Additional experiments were performed under various solution conditions known to influence ion-protein and possibly water-protein interactions. These data are compared to those obtained in different Hofmeister salts in efforts to identify changes in solvation and RecA structure.

3366-Pos Board B94
Modeling the Effects of Hydrogen-Bond Disrupting Solvents on the Structure of Model Peptide Antibiotics
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The increase in antibiotic resistant infections is a serious threat to public health. Peptide antibiotics, which can perturb the cell membrane, offer one promising solution. We are investigating the structural properties of peptide antibiotic models composed of the hydrophobic dialkylated amino acid Aib (α-aminoisobutyric acid), which imparts a strong 310-helical bias due to steric hindrance at the α-carbon. Previous studies have shown that insertion of adjacent neutral monoalkylated amino acids into an Aib sequence creates a region of the helix that is highly sensitive to disruption of hydrogen bonds by strongly hydrogen-bonding solvents. In particular, the chemical shift of the amide hydrogen at position six of the octameric peptide AA45, which has two adjacent alanines in the center of the helix, is highly sensitive to the concentration of DMSO. Smaller changes in chemical shift are also observed at position seven on the helix. In this study, we have developed a thermodynamic model that describes the solvent-enabled disruption of internal hydrogen bonds within the helix as a function of DMSO concentration. We observe that the unusual concavity of the titration curve for the amide hydrogen at position six can be modeled as the result of DMSO-driven disruption of the hydrogen bond in the presence of competing hydrogen bonds with the surrounding solvent (chloroform). In addition, we compare the results of this model with NMR structures for the corresponding Aib helix and find that the behavior of the amide hydrogen at both positions six and seven is consistent with the formation of a kink in the helix at that position.

3367-Pos Board B95
Spin-Label ESR Study of the Protein Domain Motion and Stability in the Presence of Crowding Effects
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Proteins fold and function in cellular environments that are crowded with other macromolecules. The cellular crowding effects on protein structure and stability is a key issue in molecular and structural biology. Here we report a spin-label ESR study of the T4 lysozyme protein at varying temperatures (280 ~ 338 K) as well as crowding effects (300 ~ 500 g/L of crowding agents, including glycerol, ficoll, and PVP). A doubly labeled T4 lysozyme (T4L-127/155), of which the two sites belong to two different but spatially-closed helical domains, is used as a model for the stability study. The corresponding cw-ESR spectra of the T4 lysozyme exhibit a substantial dipolar broadening at room temperature and confirm that the conformation is not disrupted by the mutations. By extracting the dipolar broadening from the spectra, this study shows a gradual decrease in the broadening with increasing temperature as glycerol or ficoll is used as a molecular crowder. Whereas, a drastic change is observed at temperatures around 330 K, which is about 10 K higher than the melting temperature of T4 lysozyme at a regular buffer, as the crowding agent is changed to PVP. The protein stability is found to increase in the presence of the PVP crowding; namely, as a consequence of the greater excluded volume effects of PVP (as opposed to those of glycerol and ficoll). Besides, an important mechanism underlying the crowding effects observed in this study probably is the glycerol/ficoll-mediated increase in solution viscosity that leads to protein domain motion inhibition.

3368-Pos Board B96
The Effect of Molecular Crowding on the Stability of Peptides
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We use Replica Exchange Statistical Temperature Molecular Dynamics (RESTMD) to investigate the effects of molecular crowding on the structure and thermodynamics of several test peptides. The test peptides are small, biologically relevant peptide that have a dominant secondary structure (either all helical or a β-barrel) and are represented via a coarse-grained computational model where the side chain - side chain interactions are based on a statistical analysis of the Protein Data Bank. The crowding agents are also peptides and are represented by the same coarse-grained model. The RESTMD algorithm naturally calculates the density of states of the system enabling us to analyze the entropic and enthalpic effects of crowding separately. The entropic effects of crowding lead to a stabilization of the test peptide relative to dilute solution. Of particular interest are the enthalpic effects, which can be either stabilizing or destabilizing. The destabilization can be large enough to overcome the entropic stabilization, resulting is a peptide that is destabilized in a crowded environment. We investigate the effect of different crowder hydrophobicities on the enthalpic contribution to the (de)stabilization of the test peptide. We show that there is a crossover temperature below which crowding agents destabilize a peptide and above which the same crowding agents stabilize it and relate this crossover temperature to the hydrophobic nature of the crowders. In addition, we will discuss the effect that crowding has on secondary structure content of the test peptides.

3369-Pos Board B97
The pH and Concentration Dependence of Protein-Protein Interactions, Conformation, and Viscosity in Crowded Protein Solutions
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Proteins are complex macromolecules with dynamic conformations that are pH- and concentration-dependent. These attributes have marked effects on solution thermodynamics and hydrodynamics (i.e. intermolecular interactions, diffusivity, and viscosity). While significant progress has been made in dilute solution hydrodynamics and thermodynamics of proteins, there is a considerable gap in our understanding of how they are altered at high concentrations. In this talk, we attempt to bridge this gap, where we present comprehensive studies of the pH and concentration dependence of conformation and viscosity of a globular protein, Bovine Serum Albumin (BSA), and an IgG1 using small-angle neutron scattering (SANS) and microfluidic rheometry, respectively. We